J. Dairy Sci. 99:1–11
http://dx.doi.org/10.3168/jds.2015-10002

Effect of duration of milk accumulation in the udder on milk composition, especially on milk fat globule

M. Dutreuil,*† J. Guinard-Flament,*† M. Boutinaud,*† and C. Hurtaud*†[1]
*INRA UMR 1348 Physiologie Environnement et Génétique pour l'Animal et les Systèmes d'Elevage (PEGASE), F-35590 Saint-Gilles, France
†Agrocampus Ouest UMR 1348 Physiologie Environnement et Génétique pour l'Animal et les Systèmes d'Elevage (PEGASE), F-35000 Rennes, France

ABSTRACT

Our objective was to study the effect of duration of milk accumulation on milk fat globules (MFG) secretion to better understand relationships between milk yield, milk fat, and MFG secretion. The modification of the milk accumulation duration in the udder is a tool to increase milk fat content. Four milking frequencies were studied on 6 dairy cows averaging 118 ± 22 d in milk: 2 milkings/d separated by 11- and 13-h or by 4- and 20-h intervals and 1 milking/d. The experimental trial was a double Latin square 3 × 3 with 2-wk periods. Postexperiment, a milking frequency of 36-h was repeated twice. Compared with 2 milkings with 11- and 13-h frequencies, 1 milking/d reduced milk and milk fat yields and increased fat content, without any effect on the size of MFG. Two milkings with 4- and 20-h intervals had no significant effect on milk fat yield and content but tended to increase the size of the MFG. Lipolysis, measured on morning milk, was weaker with 1 milking/d. When data were analyzed according to milk accumulation duration (4, 11, 13, 20, 24, and 36 h), the highest fat content and the largest diameters of MFG were obtained on milks from 4 and 36 h milkings (62.8 g/kg, 4.15 μm and 57.7 g/kg, 4.09 μm, respectively). Such observations could have 2 origins: the richness in residual milk of the 4-h milk and the coalescence of MFG related to the long milk accumulation duration in the 36-h milk. For each duration of milk accumulation, a relationship exists between MFG size and fat yield. The positive relation between MFG size and fat content was confirmed at each duration of milk accumulation. Rate of secretion of milk fat (milk accumulation of 4 h excluded) was also well correlated with MFG size. For the 36-h milk, this relationship was also observed but with a significantly different slope, assuming phenomena of MFG coalescence in response to the supposed increased intramammary pressure or to slower secretion rate and, hence, fusion events of microlipids droplets in the cytoplasm. Duration of milk accumulation joined with large increases in milk fat content induces changes in MFG size.
Key words: milk composition, milk fat, milk accumulation duration, milk fat globule

INTRODUCTION

Milk from cows is composed of about 4% of fat. Milk fat structure and composition are particularly complex, with not less than 7 different kinds of lipids (neutral glycerides, phospholipids, sphingomyelin, cerebrosids, gangliosids, cholesterol, carotenoids, and vitamin A). Milk fat is organized as milk fat globules (**MFG**), which are droplets of triglycerides surrounded and stabilized in the aqueous phase by a membrane. The latter is derived from the plasma membrane and part of the mammary epithelial cells content. Whereas numerous studies have looked at the effect of farm management practices on fatty acid synthesis (Chilliard et al., 2000, 2001), very few papers focused on the effect of farm management factors on MFG size and structure (Logan et al., 2014; Lopez et al., 2014). Likewise, whereas the consequences of a change in milk fatty acid profile on technologic, sensory, and nutritional properties of milk fat are well known (CLA or *trans* fatty acids for instance; Chilliard et al., 2001), the understanding of the influence of MFG size on those properties are lacking. Most often the effect of MFG size on milk fat properties is studied using technological treatments (centrifugation, gravitational separation, cross-flow microfiltration) to select small and large fat globules from the same mixed or individual milks (Goudédranche et al., 2000; Spitsberg, 2005; Lopez, 2011). Individual characteristics of dairy cows and feeding management can be used to adjust MFG functionalities (Couvreur and Hurtaud, 2007). For instance, selection based on individual variability could be used to modify MFG size (Couvreur and Hurtaud, 2007; Logan et al., 2014). The

Received June 23, 2015.
Accepted January 4, 2016.
[1]Corresponding author: Catherine.Hurtaud@rennes.inra.fr

same way, a change in milk fatty acid profile has been reported after a change in feeding management and, more specifically, a change of forage type or a change of the forage-to-concentrate ratio or the addition of fat in the diet (Couvreur et al., 2006, 2007; Hurtaud et al., 2010; Mesilati-Stahy et al., 2015). The effects of feeding management and individual variability on MFG size are additive, with no interaction (Couvreur and Hurtaud, 2007).

Couvreur and Hurtaud (2007) highlighted relationships between MFG size and milk composition variables such as milk proteins, calcium or the activity of fatty acids desaturation by the epithelial mammary cells. Briard et al. (2003), Couvreur and Hurtaud (2007), and Mesilati-Stahy et al. (2015) reported a positive relationship between MFG size and milk fat content. Wiking et al. (2004) showed a relationship between MFG size and only diurnal milk fat synthesis. Regarding these conflicting results, we can conclude that factors determining MFG size are not known. To study those relationships, it is necessary to modify MFG size. Milking frequency appears to be a good tool to do so, especially to modify milk fat content without changing milk fatty acid profile. Indeed, Rémond et al. (2009) demonstrated that atypical milking frequencies increased milk fat content from 30 to 65 g/kg. With milk fat content varying from 37.1 to 43.3 g/kg with milking frequencies, Delamaire and Guinard-Flament (2006a) found that changes in milk fatty acid composition were minor.

We studied the effect of atypical milking frequencies on milk fat secretion and MFG characteristics. Cows were milked twice a day, with 11 and 13 h between each milking, and once a day to study the changes in milk fat synthesis and secretion. An atypical milking frequency with 2 milkings 4 and 20 h apart was done to study milk fat properties from residual milk. Finally, the effects of a long milk accumulation in the udder were studied with a 36-h interval between milking.

MATERIALS AND METHODS

Animals

This study was conducted at the Méjusseaume experimental farm (UMR 1348 PEGASE, Le Rheu, France), and used 6 mid-lactation dairy cows. At the beginning of the experiment, their lactation stage was 117 ± 22 d. They produced 34.3 ± 3.6 kg/d of milk yield with average milk fat content of 40.0 ± 4.8 g/kg and average milk protein content of 31.8 ± 2.1 g/kg. Their BW was 637 ± 56 kg. Milk fat globule diameter varied between 3.1 and 4.3 μm, with an average of 3.8 ± 0.4 μm.

Treatments

Milking frequencies were used to describe MFG under different milk accumulation durations. Four experimental treatments were applied: 2 milkings per day at 0645 and 1745 h (durations of milk accumulation in the udder: 11 and 13 h; $\mathbf{2M_{11-13}}$); 1 milking per day at 0645 h (duration of milk accumulation in the udder: 24 h; $\mathbf{1M_{24}}$); 2 milkings per day at 0645 and 1045 h (durations of milk accumulation in the udder: 20 and 4 h; $\mathbf{2M_{4-20}}$); and, at the end of the experiment, a milking interval of 36 h ($\mathbf{1M_{36}}$) was studied twice for all the dairy cows. Dairy cows were milked on Sunday at 1745 h, on Tuesday at 0645 and 1745 h, and on Thursday at 0645 h.

Experimental Design

The experimental design was a double Latin square design with four 2-wk periods (1 wk of adaptation to treatment and 1 treatment week with measurements) with 3 milking frequencies. Between each period (Saturday and Sunday), cows were milked twice (0645 and 1745 h). For $1M_{36}$, it was a continuous design with 2 repetitions.

Feeding

The composition of the experimental diets is given in Table 1. Diets were formulated to meet energy and protein requirements of cows according to the French Unité Fourragère Lait and protein digested in the small intestine (PDI; INRA, 2007). Energy and protein balances were calculated as the differences between energy and protein supplies and energy and protein needs (INRA, 2007). All diets were isoenergetic (0.32 MJ of NE_L/kg of DM) and isonitrogenous (104 g of protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-fermented OM per kilogram of DM; INRA, 2007; PDIE). Diets contained 64.7% corn silage, 15% energy concentrate (20% wheat, 20% corn, 20% barley, 15% fine wheat bran, 20% beet pulp, 1% sunflower or rapeseed oil, 3% cane molasses, and 1% salt), 8% formaldehyde-treated soybean meal, 10% dehydrated alfalfa, 100 g of urea, and 300 g of 6% phosphorus, 24% calcium, and 4% magnesium mineral and vitamin mix as a TMR. The dairy cows were fed ad libitum.

Measurements and Sampling Scheme

Feeds were weighed, mechanically distributed twice daily at 0800 and 1700 h, and manually mixed. Volun-